

Latex Allergens in Tire Dust and Airborne Particles

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The prevalence and severity of latex allergy has increased dramatically in the last 15 years due to exposure to natural rubber products. Although historically this health risk has been elevated in hospital personnel and patients, a recent survey has indicated a significant potential risk for the general population. To obtain a wide-spread source for latex exposure, we have considered tire debris. We have searched for the presence of latex allergens in passenger car and truck tire tread, in debris deposited from the atmosphere near a freeway, and in airborne particulate matter samples representative of the entire year 1993 at two sites in the Los Angeles basin (California). After extraction of the samples with phosphate buffered saline, a modified-ELISA inhibition assay was used to measure relative allergen potency and Western blot analyses were used to identify latex allergens. The inhibition studies with the human IgE latex assay revealed inhibition by the tire tread source samples and ambient freeway dust, as well as by control latex sap and latex glove extracts. Levels of extractable latex allergen per unit of protein extracted were about two orders of magnitude lower for tire tread as compared to latex gloves. Western blot analyses using binding of human IgE from latex-sensitive patients showed a band at 34–36 kDa in all tire and ambient samples. Long Beach and Los Angeles, California, air samples showed four additional bands between 50 and 135 kDa. Alternative Western blot analyses using rabbit IgG raised against latex proteins showed a broad band at 30–50 kDa in all samples, with additional bands in the urban air samples similar to the IgE results. A latex cross-reactive material was identified in mountain cedar. In conclusion, the latex allergens or latex cross-reactive material present in sedimented and airborne particulate material, derived from tire debris, and generated by heavy urban vehicle traffic could be important factors in producing latex allergy and asthma symptoms associated with air pollution particles. *Key words:* airborne particles, *Hevea brasiliensis*, latex allergen, tire debris. *Environ Health Perspect* 104:1180–1186 (1996)

Latex allergy has become a significant health hazard since its recognition about 15 years ago (1). Latex allergy is triggered by proteins that are present not only in the latex sap from the *Hevea brasiliensis* tree, the raw material for the production of natural rubber goods, but also in many final manufactured products such as latex examination gloves, condoms, and balloons (2). Exposure to these allergens leads to production of latex-specific IgE antibodies in susceptible individuals. Symptoms of latex allergy resulting from IgE-mediated reactions are those customarily diagnosed for atopic allergic disease, including contact urticaria (itchiness, hives), rhinitis (sneezing, watery eyes, nasal congestion), asthma (inflamed constricted airways, wheezing, difficulty breathing), and anaphylaxis (decrease in blood pressure, circulatory collapse, death) (3–5). Severity can range from mild to fatal. Historically, two groups in the medical environment have exhibited an increased risk of latex allergy, presumably due to increased exposure to latex products. Health-care workers exposed frequently to latex gloves show a high incidence (9–15%) (6–8) as do patients exposed to numerous surgeries such as those suffering from spina bifida (30–65%) (9). A recent survey of blood bank sera from the general adult population in Detroit, Michigan revealed the presence of anti-latex

IgE antibody in 6.5% of the volunteers, thus indicating the potential for latex allergy in roughly 17 million individuals in this country (10).

Tire production is now the largest application for natural rubber and accounts for 75% of the global consumption (11). Although both synthetic and natural rubber are used in tire compounding, the proportion of natural rubber has increased with the conversion from bias ply to modern radial ply tires (12). The tire treads, providing the point of contact with the road, wear off to form particulate tire debris (13). This tire-wear debris has been encountered suspended in the urban atmosphere as well as deposited along the roadside (14–16). In conjunction, this information leads to the possibility of the dissemination of latex allergens in the urban environment via dispersion of tire tread debris.

In the present study, we focus on a search for the presence of latex allergens in tire tread source samples and in both sedimented and airborne particulate material in an urban environment. We first extracted latex allergens in passenger car and truck tire tread samples, as well as in a sample of particles deposited near a freeway. We then employed Western blots to identify latex allergens in these extracts and in extracts

from urban airborne particulate material collected systematically over a full annual period at two sites during the year 1993.

Methods

Sample sources: collection, preparation, and characterization. Bodyguard latex examination gloves (Cat. 7001, Lot 9306083317, TK Products Co. Ltd., Huntington Beach, CA) were selected as a reference source of latex allergen because this make has already been shown to contain intermediate levels of extractable latex allergens in a study comparing 71 separate glove lots (2). The allergen yield from the lot that we used was within 5% of the value reported by Yunginger et al. (2) when standardized to the Food and Drug Administration FDA-E5 latex reference material described below. In addition, three tire tread source samples, three ambient samples, and three synthetic rubber control samples were used in the present study. The tire tread source samples were processed in order to increase the surface area for improved protein extraction. A radial passenger car tire was run on a rolling resistance testing machine at a tire testing laboratory (16–18), and the tread of a truck tire was sanded with a belt sander to provide particles. Recap waste shavings produced in the preparation of truck tires for recapping were obtained at a local tire retreading service. Ambient particulate material deposited from the atmosphere adjacent to a curve on the Ventura-San Diego freeway interchange in Los Angeles was collected from a freeway guardrail. Ambient airborne particle samples were collected at Los Angeles and Long Beach, California for 24 hr every sixth day for the year of 1993. Course particles (>3 µm diameter) were collected using a high volume dichotomous sampler (19) at a flow rate of 300 lpm onto prebaked quartz fiber filters (2500 QAO, Pallflex Products Co.,

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Putnam, CT), which were stored frozen until utilized. For the present study, one quarter of each of the 60 filters taken at each site was used. This is equivalent to 6500 m³ of air sampled at each site. Polybutadiene and polystyrene-butadiene rubber control samples that contained no latex were prepared as thin slices using a scalpel. Non-latex tire tread was abraded with a wire brush to provide particles for a third control containing no natural rubber.

Polarized light microscopy techniques (20) were used by R. J. Lee Group Inc. (Monroeville, PA) to characterize the particle size and composition of the three tire source samples and the ambient freeway guardrail sample.

Protein extraction and determination.

Latex examination gloves cut into small pieces with scissors were extracted in plastic containers overnight, with agitation in the cold, using 5 volumes (w/v) 0.01 M phosphate, pH 7.5, containing 0.15 M NaCl and 0.02% Na azide (PBSA). In the case of the Western blot analyses, those samples that initially showed only faint bands were reextracted as before, but with the addition of 0.01% sodium dodecylsulfate (SDS) to improve extraction efficiency. After dialysis (Spectra/por 3, MWCO 3500, Spectrum Medical Industries Inc., Houston, TX) against water, the sample was freeze-dried and resuspended in PBSA. The samples were centrifuged to remove any insoluble material and stored frozen. The tire rubber source samples and freeway guardrail deposit samples were treated in the same manner. Los Angeles and Long Beach filters containing airborne particles were cut with a razor blade and only one-fourth of each filter was extracted. The 60 quarter filters for each site were extracted with 100 ml PBSA. For the purposes of comparison, a positive control sample was created consisting of latex extract prepared from the sap of the rubber tree (*Hevea brasiliensis*), collected in Malaysia. Protein concentrations were measured using the bicinchoninic acid protein assay (Sigma Chemical Company, St. Louis, MO) according to instructions of the supplier (21).

Production of antiserum against glove extract. Rabbits were used to produce polyclonal antibodies to the natural rubber proteins extracted from the latex examination gloves. For primary immunization, New Zealand white rabbits were injected with an emulsion consisting of 0.5 ml PBS containing 100 µg protein and 0.5 ml Freund's complete adjuvant into 10 subcutaneous sites. Animals were boosted three times at tri-weekly intervals using an emulsion of 100 µg protein and Freund's incomplete adjuvant. Animals were bled from the central auricular artery in the ear according to the method of

Tillman and Norman (22). Prior to immunization injections and bleeds, animals were injected subcutaneously with Innovar-Vet (Pitman-Moore Inc., Mundelein, IL), which contains an analgesic to reduce animal discomfort and eliminate the need for animal restraining devices.

Inhibition immunoassay for allergens.

Latex and other allergens were measured by inhibition of a liquid-phase immunoassay for IgE measurement (AlaSTAT, Diagnostic Products Co., Los Angeles) (23). In the initial steps of this ELISA assay, allergen-specific IgE obtained from the sera of latex-sensitive patients was allowed to react in the soluble phase with allergen present in unknown quantity in the tire dust test extracts along with a known amount of labeled allergen (allergen linked to a biotinylated dextran matrix). The allergen from the test sample and the labeled allergen compete for binding to the IgE in proportion to their respective concentrations. Only the labeled allergen-IgE complex is subsequently linked to the solid phase through biotinylated serum albumin anchored to the tube after the addition of avidin, which forms an avidin bridge between the two biotinylated moieties. By varying the ratio of the labeled to unlabeled allergen source materials, the amount of allergen in the unlabeled source can be determined through its ability to inhibit reaction of labeled allergen with the human IgE. The bound IgE is quantified by the addition of a secondary antibody (horseradish peroxidase-labeled monoclonal mouse anti-human IgE), addition of the substrates, and colorimetric detection of products. To determine latex allergen in sample extracts, the AlaSTAT assays were performed according to the supplier's instructions, except serial dilutions of the test extracts (10 µl) were added to the latex-specific patient pool serum (1:15 dilution, 50 µl) prior to the addition of 100 µl of biotin-tagged latex allergen (K82). The latex-specific sera were obtained from eight patients demonstrating a clinical sensitivity for latex and were supplied by Plasma Laboratories (Everett, WA). Each immunoassay was run in duplicate, and the standard curve was linear in the 20–80% inhibition range with an intra-assay variation (1 standard deviation) of $\pm 1.5\%$ at a level of 80% inhibition, increasing to a variation of $\pm 4.5\%$ at 20% inhibition. Inhibition tests using mountain cedar (T6) and *Dermatophagoides pteronyssinus* mite (D1) allergens together with respective allergen-specific patient pooled sera (three and five clinically sensitive patients, respectively) were performed to investigate allergen specificity.

To quantify our latex allergen results for comparison with others, a Food and Drug Administration (FDA) reference latex

preparation (FDA-E5) containing a defined activity (100,000 arbitrary units/ml) was used in the AlaSTAT latex inhibition assay to construct a standard curve. The inhibition line was linear from 20% (9.5 AU) to 80% (208.6 AU) with 1470 ng of FDA-E5 protein corresponding to 50% inhibition (44.6 AU).

Electrophoresis and immunoblotting.

Electrophoresis on pre-poured 4–20% acrylamide gels (Novex, San Diego, CA) was performed according to the method of Laemmli (24). Allergen extracts were boiled in 0.0625 M Tris, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol. The resolved components were transferred from the gel to 0.45 µm pore size nitrocellulose (Novex) in an electroblotting buffer containing 25 mM Tris (pH 8.3), 192 mM glycine, and 20% methanol for 45 min at 25V. The membranes were then washed with 0.05 M Tris containing 0.15M NaCl (TBS, pH 8.0) for 10 min and blocked by incubation with 2% polyvinyl alcohol for 1 hr at room temperature. Membranes were then probed either with human antisera for the detection of IgE-specific allergens or with rabbit anti-glove antisera for the detection of latex antigens. For detection of allergens, 5 ml of appropriate undiluted human serum (latex-sensitive pool, *Dermatophagoides pteronyssinus* mite-sensitive pool, or negative control pool) was added as the primary antibody and the membranes were incubated overnight with rocking at 4°C. After washing three times with TBS containing 0.05% Tween 20 (TBST), the membranes were incubated for 1 hr at room temperature with the secondary antibody, alkaline phosphatase conjugated mouse monoclonal anti-human IgE (Diagnostic Products Co.), diluted to 2 µg/ml in TBST containing 5% dried Carnation milk. After washing three times with TBST, the freshly prepared enzyme substrate (1.5% 5-bromo-4-chloro-3-indoyl phosphate, 3% *p*-nitro blue tetrazolium in 0.1 mM Tris, 0.15 mM NaCl, 1 mM MgCl₂, 0.01 mM ZnCl₂, 10 mM diethanolamine, pH 9.55) was added and incubated until color development was stopped by rinsing in water. In separate experiments for the detection of latex antigens, the membranes were treated with a 1:500 dilution of rabbit latex glove antiserum as a source of primary antibody and alkaline phosphatase conjugated-polyclonal goat anti-rabbit IgG as a secondary antibody.

Results

Sample particle characterization and extractable protein. Microscopic examination using polarized light techniques indicated that the three tire tread source samples were all composed of black particles of

opaque elastomer, but the estimated particle sizes varied. The truck tire, passenger car radial tire, and recapping plant waste truck tread debris contained particles of an average estimated diameter of 220 μm , 160 μm , and extremely coarse chunky sizes, respectively. The major component of the environmental freeway guardrail dust sample contained black rubber particles, equivalent to about 75% of the sample volume and showing an estimated average particle diameter of 160 μm . This sample also contained minor amounts of smaller sized 80- μm diameter quartz-feldspar particles (about 12%) and concrete particles (about 12%), as well as trace amounts of synthetic fibers, fiberglass, cellulose, plant parts, and mica-like particles.

Relative levels of extractable protein from the latex source and ambient samples are presented in Table 1 along with synthetic rubber controls that do not contain any latex. The protein yields per gram of source material processed for the tire tread source samples varied approximately sevenfold, with the highest yield being comparable to that of the latex glove sample. The protein yields per gram of material processed for the outdoor deposited and airborne particulate matter samples were almost an order of magnitude higher. Although no protein was

Table 1. Extractable protein from source and environmental samples

Samples	Extractable protein ^a , $\mu\text{g/g}$
Latex glove	455 \pm 78 (n = 2)
Car radial tire dust	395 \pm 6.6 (n = 2)
Truck tire dust	112 \pm 1.9 (n = 3)
Recap waste	59 \pm 11 (n = 3)
Freeway deposit	3259 \pm 586 (n = 4)
Long Beach airborne particles	2521; 0.148 $\mu\text{g}/\text{m}^3$ (n = 1)
Los Angeles airborne particles	3654; 0.201 $\mu\text{g}/\text{m}^3$ (n = 1)
Controls	
Butadiene polymer	ND (n = 2)
Styrene-butadiene polymer	ND (n = 2)
Non-latex tire dust	31 \pm 1.4 (n = 2)

^aProtein yield/initial sample weight or air volume as assayed by the bicinchoninic acid method and presented as the mean of one to four separate extractions for each sample \pm the standard error of the mean.

ND, below detection limit ($< 5 \mu\text{g/g}$).

detected in extracts of the synthetic rubber polymers, a small amount was extracted from the control tire dust containing no natural rubber. Tire tread may contain natural products other than latex (16). Also the colorimetric determination of protein may be an overestimate of total protein. Colloidal gold stain after acrylamide gel electrophoresis showed faint protein bands for the source tire, freeway guardrail, and airborne particulate extracts compared with latex and glove extracts that showed multiple heavy protein bands when similar protein concentrations were used (not shown).

Extractable allergens. The results of sample-extract inhibition of latex-specific IgE immunoassays used to detect and quantify soluble latex allergens are shown in Figure 1; an increase in inhibition indicates an increase in allergen. Extracts of latex sap, latex examination glove, freeway guardrail deposited dust, and three tire types clearly inhibited IgE binding, but dust mite extract did not. Inhibition curves for latex sap and latex glove extracts were essentially parallel, while the slope for the freeway dust extract was slightly steeper. Inhibitory activity for the extracts varied over a 2000-fold range when normalized with respect to the amount of protein supplied to each assay. Tenfold less latex protein and roughly 200-fold more tire and freeway dust protein were required to obtain activity comparable to glove latex protein. When standardized to the FDA's reference latex preparation E5 and then adjusted for the yield of protein extractable from the original sample (listed in Table 1), these results are equivalent to the following levels of latex allergen (microgram

of FDA latex equivalent protein per gram original weight): 629, 3.48, 1.31, 0.6, and 20.75, respectively, for latex gloves, car radial tire dust, truck tire dust, recap waste, and freeway dust. Inhibition assays were not performed using the ambient airborne particulate samples due to the small amount of material available. Extracts of butadiene polymer, styrene-butadiene polymer, and non-latex tire tread control samples (containing no natural rubber) demonstrated no inhibition (not shown).

Extract inhibition of both the mountain cedar-specific and dust mite-specific IgE immunoassays was performed to investigate allergen specificity. Results are shown in Figure 2. In the mountain cedar allergen assay, the latex sap, latex glove, guardrail dust, passenger car radial tire, and tire recapping debris extracts all demonstrated low inhibitory activity varying within a narrow range, whereas results from the dust mite allergen assay indicated very low activity for the freeway sample and no activity for the other samples.

Identification of latex allergens and antigens. In order to detect latex allergens, Western blots were probed with IgE from the pooled sera of latex-sensitive patients. Many more bands were observed in the mountain cedar, latex sap, and latex glove control extracts than for the tire and environmental extracts (Fig. 3). The molecular weight estimates supplied here in the text are accurate to about $\pm 1 \text{ kDa}$ at low molecular weights to $\pm 2.5 \text{ kDa}$ at the high molecular weights. The latex sap control showed 21 discernible bands, with the most intense bands at 40 and 46 kDa. Ten bands were observed in the glove extract control with a major

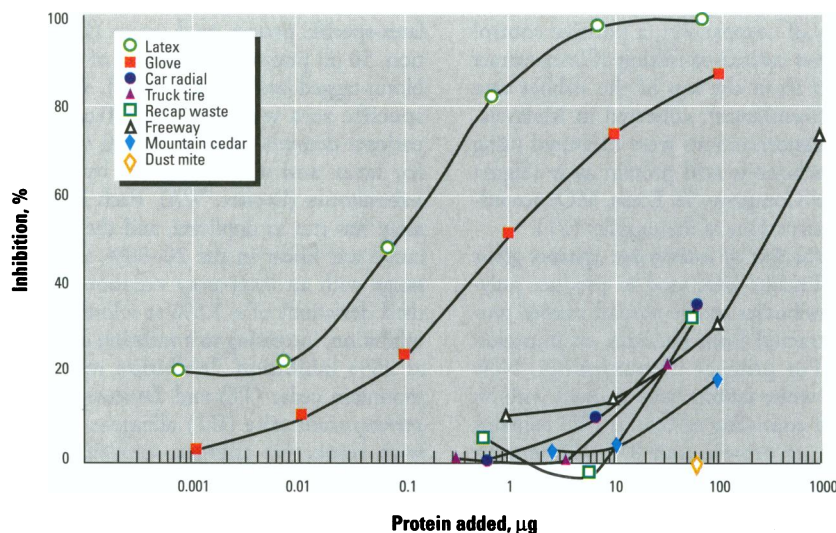


Figure 1. Inhibition of the latex immunoassay by the addition of extracts. Aliquots of extracts were added to pooled sera from latex-sensitive patients to allow the competitive binding of the specific IgE before addition of the latex assay allergen, as described under Methods.

band at 45 kDa. A 34–36 kDa band was observed for all tire and environmental samples. Additional bands were present in the truck tire (59 kDa), passenger car radial tire (51, 63, 78 kDa), and Long Beach (50, 65, 82, 99 kDa) and Los Angeles (60, 73, 88, 135 kDa) atmospheric particle samples. It is noteworthy that no bands were encountered for the control extraction blank probed with human anti-latex IgE. No bands were found for the butadiene, styrene–butadiene, or non-latex tire tread control samples (not shown). The negative control blots, probed with IgE-stripped sera or sera from patients sensitive to house dust mite, showed no binding. Unexpectedly, at least 11 proteins binding IgE were found in the mountain cedar control, with major bands at 27–30, 42, and 73 kDa. When the individual sera used to make the latex positive pool were tested for specific IgE to mountain cedar in the AlaSTAT assay, six of the eight sera were positive.

The extracts were further characterized by Western blot analysis with polyclonal IgG rabbit antisera prepared by immunization of rabbits with the latex glove extract (Fig. 4). One major band (42 kDa) and six very minor bands ranging from 21 to 92 kDa were observed when the mountain cedar extract was probed with the rabbit anti-latex protein sera. Major bands at 27, 39, and 45 kDa and 11 minor bands were visible for the latex sap sample. The glove extract showed major bands at 46 and 52 kDa and eight minor bands. All environmental samples showed reactivity in the broad range of 34–56 kDa, and additional bands were visible when the Long Beach (65, 82, 99 kDa) and Los Angeles (76, 88 kDa) airborne particle sample extracts were probed with the latex-sensitive rabbit antisera.

Discussion

Results presented above revealed the presence of extractable latex allergens not only in tire treads but also in ambient samples of sedimented freeway dust and airborne particulate matter from two locations within the Los Angeles basin. Initially, three tire tread source samples were extracted with phosphate buffered saline. Higher protein yields per gram of source material processed were obtained in the extraction of smaller tire tread particles having a higher surface area. This result is consistent with the low solubility of the hydrophobic rubber material in which increased surface exposure to the aqueous solvent would be expected to improve extraction. The protein yields per gram of material processed for the airborne particulate matter samples were almost an order of magnitude higher than for bulk tire dust. Likewise, this may be expected due to the generally smaller particle size of the sus-

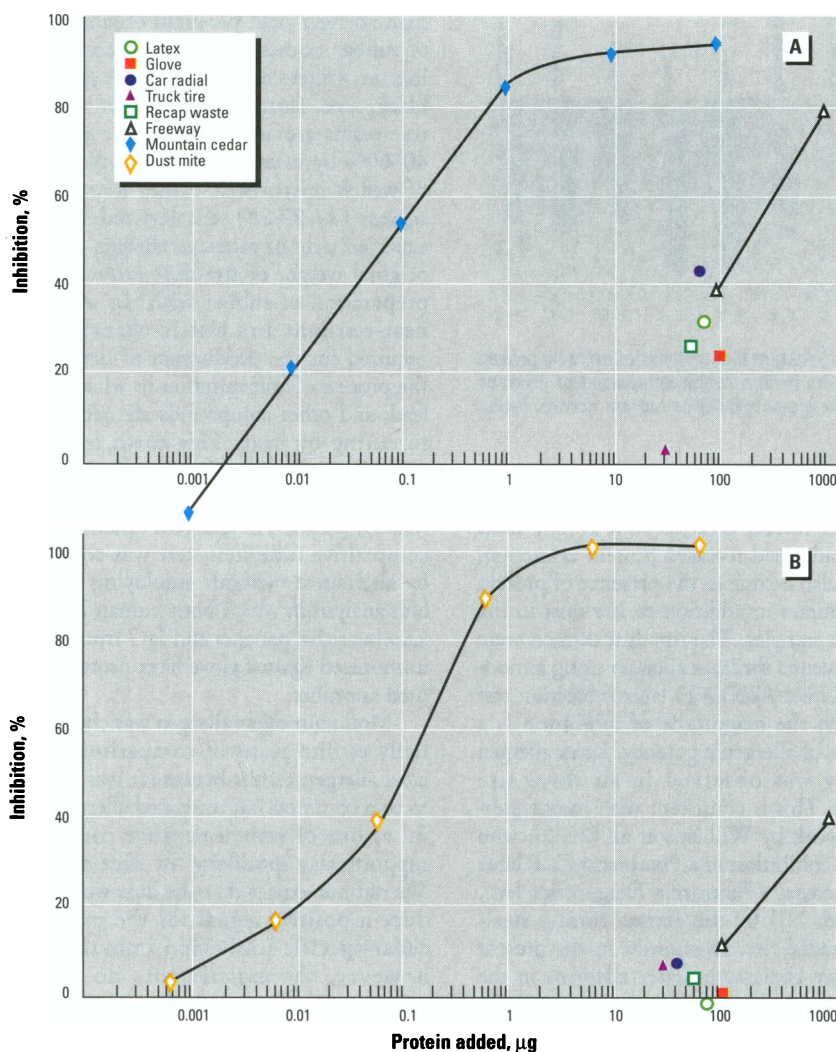


Figure 2. Inhibition of (A) mountain cedar or (B) dust mite immunoassays by the addition of extracts. Aliquots of extracts described in Figure 1 were added to pooled sera from mountain cedar-sensitive patients or dust mite-sensitive patients prior to the addition of the respective mountain cedar or dust mite assay allergen to permit the competitive binding of specific IgE.

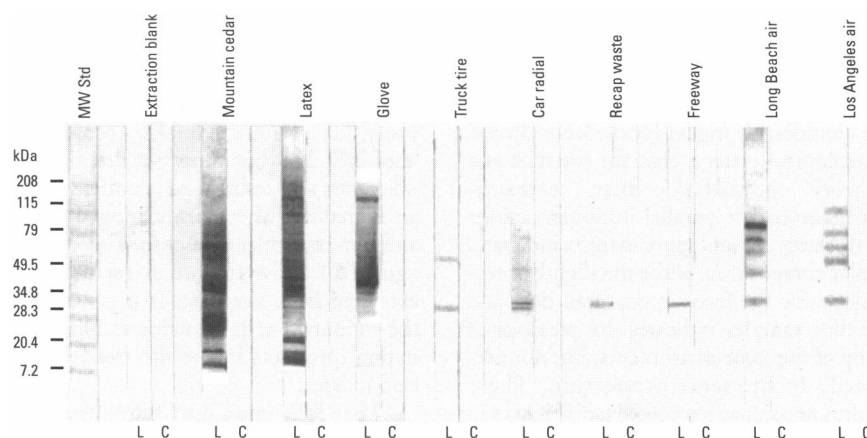


Figure 3. Western blot analysis of extracts probed with IgE from pooled sera from latex-sensitive patients (L) or from a negative control pool of human IgE-stripped sera (C). MW std, molecular weight standards.

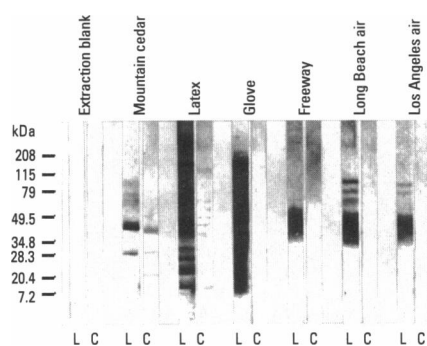


Figure 4. Western blot analysis of extracts probed with serum from a rabbit immunized to produce anti-latex glove IgG (L) or normal control rabbit sera (C).

pended particulate matter samples, yielding a greater surface area per unit sample mass that would lead to eased protein extraction. It may also be due to the presence of protein from sources in addition to tire dust in the ambient samples. The tire dust extracts were next screened for latex allergen using a modified ELISA (AlaSTAT) latex inhibition test in which the magnitude of inhibition is a measure of allergenic potency. Latex allergen activity was observed in all three tire extracts. This is consistent with recent published work by Williams et al. (25,26) who showed inhibition of a Pharmacia CAP latex immunoassay (Pharmacia Diagnostics Inc., Fairfield, NJ) by the extract from a steel-belted radial tire. In contrast in the present study, we characterize latex allergens in the environment through the systematic examination of a sample of particulate matter deposited from the atmosphere adjacent to a curve on a freeway connection and through a complete 1-year air monitoring experiment in two major southern California cities. The extract of the freeway guardrail dust sample inhibited *in vitro* IgE-latex binding with a dose-response equivalent to that for tire dust samples. That the activities were similar to those of tire dust was encouraging as this particular ambient sedimented freeway dust sample contained 75% by mass of rubber debris. The latex allergen activity was considerably higher for the latex sap and glove control extracts than for tire dust and freeway guardrail dust extracts. Furthermore, the parallel inhibition curves for the latex sap and glove extracts indicate a similar composition, while the slightly steeper slope for the freeway guardrail dust and tire dust samples indicates the presence of many of the same constituents, but not necessarily in the same proportion. These results are compatible with the differences in fabrication processes and formulations for dipped latex products versus tire tread. Natural rubber latex accounts for over 90%

of dipped product content and therefore a high allergen yield per gram original weight of rubber extracted. Tire tread compounding, in which components such as carbon black, zinc, stearic acid, paraffin wax, and tree resins are added, leads to only about 40–60% by mass of rubber polymer, much of which is synthetic rather than natural rubber (11,27,28), thereby reducing the expected yield of extracted allergen per gram original weight of tire dust extracted. The preparation of rubber with the enhanced heat-resistant and elastic characteristics required for the production of tires entails the process of vulcanization in which sulfur, lead, and other compounds are added prior to curing by heat. This harsh treatment could be expected to destroy proteins in the natural rubber. It is noteworthy, however, that the presence of allergens observed in the competitive inhibition test was confirmed by alternative methods employing Western blot analysis in which both human IgE from latex-sensitive patients and IgG from rabbits immunized against glove latex proteins were used as probes.

Mountain cedar allergen was chosen initially as one point of comparison to the latex allergen results because it was thought to be a common but unrelated allergen, useful as one of several negative controls to support assay specificity for latex allergens. We did not expect that tire dust would produce a positive signal for the mountain cedar-specific inhibition immunoassay; however, the experiments do show a response. The unexpected result could be explained by three possibilities. First, there could be a cross-reaction between latex and mountain cedar in which similar or identical proteins are found in both allergen sources. Cross-reactivity has already been demonstrated between latex and banana, kiwi, chestnut, avocado, and peanut (29,30). Our results using IgG from rabbits specifically immunized with latex glove extract showed the presence of cross-reactive material in mountain cedar extracts in the Western blots. Second, the mountain cedar-sensitive patient pool could contain individuals whose sera also contain anti-latex IgE. Third, it turns out that tire manufacturers add resins (e. g., conifer pitch) as an ingredient when formulating tire tread, and our experiments published several years ago (16) show that such resins can be extracted from tire dust. It is possible that the mountain cedar reaction is induced by natural products in the tire tread in addition to latex.

That IgE house dust mite interactions were not blocked by tire extracts supports the specificity of the tire dust and airborne particle sample reactions with latex IgE

antisera. Also non anti-latex IgE controls consisting of dust mite or IgE-stripped patient antisera did not react with latex allergens in the Western blot assays.

Our data from the Western blot analyses indicate the presence of several latex allergens in the environment. The major band of IgE reactive allergen occurring at 34–36 kDa in the tire dust and freeway dust extracts, as well as in the airborne particle samples, suggests tire tread as a source. The four higher molecular weight bands of 50–135 kDa found in the airborne particle samples, but not in our tire extracts, could be from tires not studied in the present investigation or from other sources including cross-reactive material from other plants. Because the anti-latex IgE-positive pooled antisera could have contained IgE to other allergenic proteins, we tested rabbit antisera prepared against latex glove extract. Although a much broader band of reaction was found at the 34–56 kDa region, the finding that this rabbit IgG anti-latex antisera recognized similar components to those found with human IgE is strong evidence for the presence of latex or at least latex cross-reactive material in the samples. Latex cross-reactive materials other than latex proteins cannot be ruled out because tire tread formulations are known to contain natural products in addition to latex that include conifer resins, among other compounding ingredients (16).

Turjanmaa (31), Fuchs and Wahl (32), Slater et al. (33–35), and others (36–38) have studied extracts of natural rubber latex, latex gloves, condoms, and balloons to identify the relevant allergenic proteins. By using Western blot analyses to identify human IgE bound to electrophoresis proteins, well over 50 allergenic bands have been reported, with major bands occurring in the 14 and 30 kDa regions (39). Recent studies have shown that the sera of several food-sensitive and latex-sensitive patients recognize a 30 kDa allergen (30). Furthermore, a 36 kDa allergen, frequently encountered by sera of patients with latex allergy (40), has been purified and shows homology to plant endo 1,3- β -glucosidases (41). Whether any of these allergens are similar to the 34–36 kDa allergen isolated from tire dust extracts, freeway dust extracts, and airborne particulate extracts in our study requires further study.

Our data showing that allergens, reactive with human IgE and rabbit IgG antibodies, can be extracted from tire tread fragments and ambient atmospheric particles raises the possibility for the exposure of large urban populations to latex allergens or latex cross-reactive materials, leading to potential health effects. Previous work has shown that over 5 tons of rubber particles with an aerodynamic

diameter less than 10 μm (PM_{10}) are released into the air of the Los Angeles basin daily (42,43). The relative amount of tire-derived PM_{10} in the atmosphere is about 1–2% of the PM_{10} particulate mass in the Los Angeles atmosphere, leading to a PM_{10} tire dust concentration of approximately 1 $\mu\text{g}/\text{m}^3$. Thus, on a daily basis, we estimate that microgram amounts of rubber particles are inhaled into the lungs and nasopharynx. Depending on the rate of removal of these particles by the mucociliary system of the airways, sufficient accumulation of rubber dust could occur to provide allergenic stimulation of IgE antibody formation or sensitization. Therefore, we wished to compare ambient outdoor exposure levels for airborne latex allergen with occupational indoor levels encountered in a study carried out in a hospital where powdered latex gloves were used (44). Even though quantitative inhibition assays were not performed on the limited amounts of airborne particulate matter samples in the present study, a lower limit estimate for an outdoor airborne latex allergen concentration of 0.028 ng FDA equivalent latex allergen/ m^3 air can be obtained from our results by using the latex allergen content of the ambient freeway dust adjusted for its 75% rubber particle content (27.7 μg FDA latex allergen/g rubber particles) in conjunction with the PM_{10} tire dust concentration in Los Angeles (1 $\mu\text{g}/\text{m}^3$). It is important to emphasize that this estimate is a lower limit due to the decreased extraction efficiency of latex protein in the rather large 160 μm freeway dust particles as compared to the <50 μm airborne particulate matter. In comparison, indoor airborne latex allergen levels in the hospital examined by Swanson et al. (44) were much higher than the average outdoor Los Angeles ambient level. When expressed in nanograms of FDA equivalent latex allergen per cubic meter of air, the levels were 104–1664 and 2.4–14.4, respectively, in areas of the hospital where powdered gloves were used and where they were seldom used (44).

Could exposure to latex allergens in outdoor ambient air trigger serious health effects? The evidence is not conclusive, but the possibility exists. What about the ambient exposure level? The lower limit annual average ambient latex allergen concentration of 0.028 ng/ m^3 estimated above at community air monitoring sites is definitely lower than expected near freeways, on days with higher than average pollutant concentrations, or during the morning traffic peak. Peak ambient exposures are at least a factor of 10 higher, in the vicinity of FDA latex allergen equivalent 0.3 ng/ m^3 . Can nanogram per cubic meter quantities of allergen induce asthma attacks? In the

hospital study (44), a technician sensitive to latex allergen did report an asthma episode at an airborne concentration of 96 ng FDA equivalent latex allergen/ m^3 . In a crab processing factory, which had concentrations of airborne crab antigen in the range 9–115 ng/ m^3 , 34% of the workers demonstrated asthmatic symptoms and 52% had crab-specific IgE antibodies (45). The outdoor latex allergen levels that we estimate are a bit lower than the above occupational exposures known to induce asthma attacks, but not so much lower as to rule out the possibility of an effect. Further study is warranted.

Asthma morbidity and mortality have been increasing in the last 20 years (46–50), and evidence exists that PM_{10} air pollution particles may be an important factor in asthma (51,52). Furthermore, a role for vehicles could be implied from results of an epidemiological study in Birmingham, United Kingdom, showing that children admitted to the hospital for asthma were more likely to live near major roads with high traffic flow than were the two control groups (children admitted to the hospital for nonrespiratory causes or children from the general population) (53). In conclusion, we suggest that the latex allergens or latex cross-reactive material present in sedimented and airborne particulate material, derived from tire debris, or generated by heavy urban vehicle traffic could be important factors in producing latex allergy and asthma symptoms associated with air pollution particles.

REFERENCES

- Nutter AF. Contact urticaria to rubber. *J Dermatol* 101:597–598 (1979).
- Yunginger JW, Jones RT, Fransway AF. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol* 93:836–842 (1994).
- Seaton AB, Cherrie B, Turnbull J. Rubber glove asthma. *BMJ* 296:531–532 (1988).
- Carrillo T, Cuevas M, Munoz T, Hinojosa M, Moneo I. Contact urticaria and rhinitis from latex surgical gloves. *Contact Dermatitis* 15: 69–72 (1986).
- Slater JE. Rubber anaphylaxis. *N Engl J Med* 320:1126–1130 (1989).
- Berky ZT, Luciani WJ, James WD. Latex glove allergy. A survey of the U.S. Army Dental Corps. *JAMA* 268:2695–2697 (1992).
- Lagier F, Badier M, Martigny J, Charpin D, Vervloet D. Latex as aeroallergen. *Lancet* 336: 516–517 (1990).
- Iacobelli AM, McCullough JA, Ownby DR. The prevalence of latex allergy in high risk medical personnel. *J Allergy Clin Immunol* 91:216 (1993).
- Alenius H, Palosuo T, Kelly K, Kurup V, Renuala T, Makinen-Keijunen S, Turjanmaa K, Fink J. IgE reactivity to 14-kd and 27-kd natural rubber proteins in latex-allergy children with spina bifida and other congenital anomalies. *Int Arch Allergy Immunol* 102:61–66 (1993).
- Ownby DR, Ownby HE, McCullough JA, Shafer AW. The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors. *J Allergy Clin Immunol* 93:282 (1994).
- Barbin WW, Rodgers MB. The science of rubber compounding. In: *Science and technology of rubber* (James EM, Burak E, Eirich FR, eds). New York:Academic Press, 1994:419–469.
- Barnard D, Baker CSL, Wallace IR. Natural rubber compounds for truck tyres. *NR Technology* 16:19–26 (1985).
- Dannis ML. Rubber dust from the normal wear of tires. *Rubber Chem Technol* 47:1011–1037 (1974).
- Pierson WR, Brachaczek WW. Airborne particulate debris from rubber tires. *Rubber Chem Technol* 47:1275–1299 (1974).
- Kim MG, Yagawa K, Inoue H, Lee YK, Shirai T. Measurement of tire tread in urban air by pyrolysis-gas chromatography with flame photometric detection. *Atmos Environ* 24A: 1417–1422 (1990).
- Rogge WF, Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT. Sources of fine organic aerosol. 3. Road dust, tire debris, and organometallic brake lining dust: roads as sources and sinks. *Environ Sci Technol* 27:1892–1904 (1993).
- Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT. Quantitative characterization of urban sources of organic aerosol by high-resolution gas-chromatography. *Environ Sci Technol* 25:1311–1325 (1991).
- Hildemann LM, Markowski GR, Cass GR. Chemical composition of emissions from urban sources of fine organic aerosol. *Environ Sci Technol* 25:744–759 (1991).
- Solomon PA, Moyers JL, Fletcher RA. High-volume dichotomous virtual impactor for the fractionation and collection of particles according to aerodynamic size. *Aerosol Sci Technol* 2:455–464 (1983).
- McCrone WC, Draftz RG, Delly JG. The particle atlas. Ann Arbor, MI:Ann Arbor Science, 1967.
- Stoscheck CM. Quantitation of protein. In: *Methods in enzymology*, vol 182 (Deutscher MP, ed). New York:Academic Press, 1990: 50–68.
- Tillman P, Norman C. Droperidol-fentanyl as an aid to blood collection in rabbits. *Lab Anim Sci* 33:181–182 (1983).
- Ownby DR, McCullough J. Testing for latex allergy. *J Clin Immunology* 16:109–113 (1994).
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685 (1970).
- Williams PB, Buhr MP, Weber RW, Volz MA, Koepke JW, Selner JC. Latex allergen in respirable particulate air pollution. *J Allergy Clin Immunol* 95:88–95 (1995).
- Williams PB, Akasawa A, Dreskin S, Selner JC. Respirable tire fragments contain specific IgE binding and bridging latex antigens. *J Allergy Clin Immunol* 95:152 (1995).
- Reisch MS. Rubber chemicals and additives adapt to changing demands. *Chem Eng News* 72(35):11–16 (1994).
- Bhowmick AK, Hall MM, Benarey HA. Rubber products manufacturing technology. New York:Marcel Dekker, 1994.
- Hovanec-Burns D, Ordenez M, Corrao M, Enjamuri S, Unver E. Identification of another latex cross reactive food allergen: peanut. *J*

- Allergy Clin Immunol 95:150 (1995).
30. Lavaud F, Prevost A, Cossart C, Guerin L, Bernard J, Kochman S. Allergy to latex, avocado pear and banana: evidence for a 30 kd antigen in immunoblotting. *J Allergy Clin Immunol* 95: 557-564 (1995).
 31. Turjanmaa K. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 17:270-275 (1987).
 32. Fuchs T, Wahl R. Immediate reactions to rubber products. *Allergy Proc* 13:61-66 (1992).
 33. Slater JE, Mostello LA, Shaer C, Honsinger RW. Type I hypersensitivity to rubber. *Ann Allergy* 65:411-414 (1990).
 34. Slater JE, Trybul DE. Affinity purification of latex antigens. *J Allergy Clin Immunol* 93: 644-649 (1994).
 35. Slater JE, Chabra SK. Latex antigens. *J Allergy Clin Immunol* 89:673-678 (1992).
 36. Jaeger D, Kleinhaus D, Czuppon AB, Baur X. Latex specific proteins causing immediate-type cutaneous, nasal, bronchial, and systemic reactions. *J Allergy Clin Immunol* 89:759-768 (1992).
 37. Alenius H, Kurup V, Kelly K, Palosuo T, Turjanmaa K, Fink J. Latex allergy: frequent occurrence of IgE antibodies to a cluster of 11 latex proteins in patients with spina bifida and histories of anaphylaxis. *J Lab Clin Med* 123:712-720 (1994).
 38. Alenius H, Makinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Allergen and protein content of latex gloves. *Ann Allergy* 73:315-320 (1994).
 39. Hamnan CP. Natural rubber latex protein sensitivity in review. *Am J Contact Dermatitis* 4: 4-21 (1993).
 40. Alenius H, Turjanmaa K, Makinen-Kiljunen S, Reunala T, Palosuo T. IgE immune response to rubber proteins in adult patients with latex allergy. *J Allergy Clin Immunol* 93:859-863 (1994).
 41. Alenius H, Kalkkinen N, Lukka M, Reunala T, Turjanmaa K, Yip E, Makinen-Kiljunen S, Palosuo T. Prohevein from rubber tree is a major latex allergen. *J Allergy Clin Immunol* 95:155 (1995).
 42. Cass GR, Boone PM, Macias ES. Emissions and air quality relationships for atmospheric carbon particles in Los Angeles. In: *Particulate carbon-atmospheric life cycle* (Wolff GT, Klimisch RL, eds). New York: Plenum Press, 1982.
 43. Gray HA. Control of atmospheric fine primary carbon particle concentrations, [PhD thesis]. California Institute of Technology, Pasadena, CA, 1986.
 44. Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, Reed CE. Quantification of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 94:445-451 (1994).
 45. Griffin P, Roberts FP, Topping MD. Measurement of airborne antigens in a crab processing factory. *Ann Occup Hyg* 38:923-926 (1994).
 46. Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, Speizer FE. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 329:1753-1759 (1993).
 47. Weiss KB, Wagener DK. Changing patterns of asthma mortality. *JAMA* 294:1683-1687 (1990).
 48. Williams MH. Increasing severity of asthma from 1960-1987. *N Engl J Med* 320: 1015-1016 (1989).
 49. Sly RM. Changing asthma mortality. *Ann Allergy* 73:259-268 (1994).
 50. Pope CA III, Dockery DW. Acute health effects of PM₁₀ pollution on symptomatic and asymptomatic children. *Am Rev Respir Dis* 145: 1123-1128 (1992).
 51. Pope CA III, Dockerty DW, Spengler JD, Raizenne ME. Respiratory health and PM₁₀ pollution. *Am Rev Respir Dis* 144:668-674 (1991).
 52. Roemer W, Hoek G, Brunekreef B. Effect of ambient winter air pollution on respiratory health of children with chronic respiratory symptoms. *Am Rev Respir Dis* 147:118-124 (1993).
 53. Edwards J, Walters S, Griffiths RK. Hospital admissions for asthma in preschool children: relationship to major roads in Birmingham, United Kingdom. *Arch Environ Health* 49: 223-227 (1994).

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